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RAPID PRECLINICAL SCREENING OF CHEMOTHERAPEUTIC AGENTS EFFECTIVE IN COMBINATION WITH IMMUNOTHERAPY

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Therapeutic alternatives are extremely limited for patients with disease relapse following allogeneic marrow transplantation. One approach has been to intensify the immunotherapy of transplantation by infusion of lymphocytes upon disease relapse (delayed lymphocyte infusion, DLI). Administration of DLI can be limited by graft versus host disease, and its effectiveness by tumor accessibility, suppressive local tumor environment, low tumor antigenicity, and systemic immune tolerance. Recent studies have shown that certain chemotherapeutic agents, especially those with known molecular targets, can be effective in combination with DLI to eliminate residual tumor. To assess the effectiveness of targeted chemotherapeutic agents in conjunction with DLI, we have developed a series of in vitro and in vivo murine assays to screen potential agents for combination DLI therapy. In vitro tissue culture with flow cytometric analysis using annexin, propidium iodine, and CFSE markers is used to establish tumor susceptibility and effective dose for each chemotherapeutic agent. Characterization of adverse in vivo effects on relevant immune effector and regulatory populations, such as memory and naïve T, antigen presenting, NK and Treg cells is then assessed using drug titration in a thymectomized mouse model to avoid the variable of thymic derived T cell renewal. Finally, optimized drug concentrations are used in an allogeneic graft versus tumor (GVT) model to screen for additive or synergistic effects on tumor growth in conjunction with DLI. Using these screening methods we have been able to rapidly identify useful chemotherapeutic agents, and establish drug concentrations capable of improving survival over GVT effects associated with DLI alone. In addition, in vitro drug effectiveness, as measured by cell membrane injury, cell death and decreased cell proliferation, has been an effective tool to predict in vivo anti-tumor responses.

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TREATMENT OF STEROID-REFRACTORY ACUTE GRAFT-VERSUS-HOST DISEASE WITH BASILIXIMAB AND ETANERCEPT IN EARLY PERIOD

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Background: Severe acute graft-versus-host disease (aGVHD) is a life-threatening complication after allogeneic hematopoietic stem cell transplantation (allo-HSCT). Steroid-resistant aGVHD(SR-aGVHD) is especially associated with a high incidence of mortality.
Methods: We performed a prospective study in treatment of grades III-IV SR-aGVHD in early period with a combination of anti-cytokine therapy composed of IL-2 receptor antibody (basiliximab) and TNF-receptor fusion protein (etanercept) from June 2009 to Agust 2010. Seven patients experienced grade IV SR-aGVHD (skin involved in seven patients, gastro-intestinal involved in six patients, liver involved in four patients). The median age was 23 years (range 11-48). Donor types were HLA-matched related (n = 1), HLA-matched unrelated (n = 2), and haploidentical related (n = 4). Anti-cytokine therapy consisted of intravenous basiliximab 20mg on days 1, 4, 8, 15, and etanercept 25 mg subcutaneous twice a week for 4 weeks, followed by once weekly for 4 more weeks.
Results: All patients (100%) achieved complete remission. Five of 7 (71.4%) patients are currently alive with a median follow-up of 163 (80–348) days after transplantation, and a median follow-up of 121 (48–321) days after Anti-cytokine therapy. Only two patients died of pulmonary infection and cardiac arrest. In our retrospectivly studied in twenty-three patients with SR-aGVHD who reveiving allo-HSCT from HLA-matched related donor (n = 1), HLA-mismatched related donor (n = 1),HLA-matched unrelated donor (n = 6), HLA-misatched unrelated donor (n = 15), the treatment of SR-aGVHD included high dose methylprednisolone, anti-thymocyte globulin, basiliximab or etanercept, respectively. Only five (21.7%) patients achieved CR and two(8.69%) achieved partial remission. Only five of 23 (21.7%) patients are currently alive with a median follow-up of 52 (32–3697) days after transplantation.The incidence of treatment-related mortality (TRM) was 78.3%. The cause were infectious complications and organ failure.
Conclusion: Inflammatory cytokines, IL-2 and TNF-a, play critical roles in the pathological of GVHD, and may be effective targets for therapy. Our data suggested that the combination of basiliximab and etanercept is well tolerated and can induce a high response rate, high survival rate with low incidence of TRM in patients with severe SR-aGVHD in early period, particularly in sever GrmonaryI aGVHD.

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MYELOABLATIVE UNRELATED CORD BLOOD TRANSPLANTATION FROM GRAFTS WITH THREE HLA ANTIGEN MISMATCHES RESULTED IN SUPERIOR OUTCOMES FOR PATIENTS WITH DE NOVO ACUTE LEUKEMIAS

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We studied the clinical outcomes of 87 adults with de novo acute leukemias who received unrelated cord blood transplantation (CBT) after myeloablative conditioning. Between August 1998 and July 2008, 55 patients with acute myelogenous leukemia (AML) and 32 patients with acute lymphoblastic leukemia (ALL) were treated with unrelated CBT. All patients received 4 fractionated 12 Gy total body irradiation (TBI) and chemotherapy as myeloablative conditioning and cyclosporine plus short term methotrexate as graft-versus-host disease (GVHD) prophylaxis. The median age was 38 years and the median number of nucleated cells was 2.38 x 10^7/kg. 16 patients were transplanted not in complete remission (CR). t(9;22) and 11q23 abnormalities were found in nine and seven, respectively. All patients received a single cord blood unit and grafts were selected from at least 4/6 serologically matched units in the Japan Cord Blood Bank Network. Variables considered in statistical analyses were age, gender, diagnosis, disease status at CBT, disease risk, cytogenetic subgroups, total nucleated cell (TNC) dose, sex mismatches, ABO mismatches, HLA mismatches (A and B by low resolution, DRB1 by high resolution), and blood levels of cyclosporine. With a median follow-up of 42 months (range 13-120), the probability of disease free survival (DFS) at 5 years was 67.1% (95%CI: 57.0-77.2%). The 5-year cumulative incidence of relapse was 23.1% (95%CI: 13.7-32.5%). In multivariate analysis, the risk factor identified for both DFS and the incidence of relapse was the numbers of HLA mismatches. The probability of DFS and the cumulative incidence of relapse in the subset of three HLA antigen mismatches (n = 25) were 89.6% and 9.9%, respectively. Several reports have shown that HLA matched CBT resulted in better outcome, but our study revealed superior outcome in recipients of grafts with three HLA antigen mismatches. Although this is a result from a Japanese single institute, we suggest that CBT from grafts with three HLA antigen mismatches may improve outcomes by reducing the incidence of relapse without increasing treatment related mortality (TRM) in adult patients with de novo acute leukemias.

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SINGLE DOSE ADMINISTRATION OF ECP TREATED CELLS PRIOR TO TRANSPLANTATION SIGNIFICANTLY INCREASES SURVIVAL IN A MHC-MISMATCHED MODEL OF ACUTE GVHD

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Extracorporeal photophoresis (ECP) has shown promise as a therapeutic modality in treating steroid-resistant chronic and acute GvHD. The way ECP mediates its effect has not been clearly elucidated, but some of the proposed mechanisms include impaired maturation of dendritic cells and reduced antigen presentation induced by apoptotic cells, as well as increased regulatory T (Treg) cells. We have developed a pre-emptive murine model of ECP to inhibit the initiation phase of acute GvHD and improve survival.
Methods: Apoptosis was induced by ECP using 8-Methoxypsoralen and UVA light (UVAR light set, Therakos). Two days prior to bone marrow transplantation (BMT), 10^7 syngeneic (BalbC; $H2^{-d}$) or allogeneic (C57BL6; $H2^{-b}$) splenocytes treated with ECP were infused into BalbC recipients. To induce acute GvHD mice received 0.75x10^6 conventional T cells (Tcon) and 5x10^6 T cell depleted bone marrow from C57BL6 ($H2^{-b}$). Animals were followed for survival, and GvHD was scored using standard criteria. In some experiments, Tcon were derived from luciferase-transgenic animals, allowing for quantification of T cell proliferation using bioluminescent imaging (BLI).